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A STUDY OF THE IN VIVO AND IN VITRO BEHAVIOR OF THE MONOCYTES OF THE BLOOD STREAM AND CONNECTIVE TISSUE

DAVID L. REEVES

From the Department of Embryology, Carnegie Institution of Washington

Submitted for publication July 1, 1934

For many years histologists have recognized a number of phagocytic cells. Inasmuch as different environments have tended to bring about certain morphological peculiarities in these cells, they have been named and classified differently in the various tissues and organs in which they were located.

The clasmatocyte, for example, is defined as an elongated, branched, irregular cell with an elliptical nucleus and granulated or vacuolated protoplasm located in the connective tissue. The Kupffer cell, often containing ingested red blood cell fragments, iron and pigment, is a highly phagocytic cell associated closely with the endothelial wall of the sinusoids of the liver. The dust cell is a large mononuclear phagocyte of the lungs which contains carbon particles in its interior. In addition to these there are many similarly classified phagocytes, such as the splenocytes, the adventitial cells, and the pyrrhol cells.

The epithelioid cell, sometimes called the endothelioid cell, is not often described as a phagocyte although it is sometimes said to contain one or more tubercle bacilli. This cell is classified as a large protoplasmic cell resembling an epithelial cell. It is observed in diseases of the blood-forming organs and in tuberculosis. The lepra cell, found in leprous nodules, is described as a cell similar to the epithelioid cell.

The origin of all these cells has been the subject of many investigations and the literature dealing with this question has been reviewed many times (Mallory, Foot, Aschoff, Sacks, Sabin and Hall). The consensus of opinion seems to originate the macrophages from leucocytes emerging from the blood stream, but the question of the origin of these cells in the blood stream has been the subject of controversy,

some investigators claiming that they arise from the reticulum of blood-forming organs, others that they come from the endothelium of blood vessels, and still others that they differentiate from pre-existing mononuclear white blood cells.

In an investigation of the subject by means of supra-vitally stained living cells, Sabin (29, 31, 32), Sabin, Cunningham and Doan (30) found that the connective-tissue macrophage (clasmatocyte) and the epithelioid cells of tuberculous lesions stained in a characteristic manner with neutral red, and that in supravitally stained films of blood studied in the warm box for short periods of time there appeared two types of stained large mononuclear cells. One of these having large stained bodies, irregularly placed, adjacent to the nucleus and resembling the stained bodies of the clasmatocytes was therefore called by these investigators a "clasmatocyte," while another type of cell with small, stained bodies arranged in the form of a rosette around the centrosphere located at one side of the nucleus they termed the "monocyte." Other cells with fewer neutral red bodies were identified as lymphocytes.

On the basis of these studies, Sabin and her co-workers claimed that there were present in the blood stream two types of large mononuclear white blood cells, one that resembled the clasmatocyte and gave rise to the tissue clasmatocyte and the other, the monocyte, that differentiated into the epithelioid cell.

Within recent years, however, other workers, Bloom (2), Cappell (3), Carrel and Ebeling (4), Hall (16), the Lewises (19, 20), and Simpson and Deming (34), have shown that the supra-vital staining methods fail to differentiate clearly between the clasmatocyte and monocyte, and inasmuch as they find the monocytic reaction to be neither constant nor characteristic, they have concluded that these cells represent merely different stages of the same cell.

In tissue cultures of the buffy coat and the hemopoietic organs it was possible, not only to demonstrate Sabin's two types of vitally stained cells (Carrel, Erdmann, Hetherington, the Lewises, Maximow, and Timofejewsky), but in hanging drops of bloods of all vertebrate classes (Lewis, and Lewis and Lewis) to follow the changes of the monocytes into macrophages and epithelioid cells similar to those of the fixed tissues, not only on the basis of the staining reaction but also on their

morphological structure. These investigators found, however, that a certain length of time was required for this transition.

In view of the fact that the investigations so far carried out had each been based on one type of experimental procedure, the importance of undertaking a study of this problem based on a comparison of the behavior of the phagocytic mononuclear cells of the blood and of the connective tissue under various experimental conditions both in the animal and in tissue culture and to observe individual cells through their transition led to the following observations and conclusions.

METHODS AND OBSERVATIONS

Washed rabbit erythrocytes (1 cc.) were injected subcutaneously into the flanks of young white rats and material was removed from the injected area at various intervals for the preparation of spreads, paraffin sections, and cultures.

A little Locke's solution in a capillary pipette was mixed with the tissue of the injected area, the loose cells were withdrawn, spread out on a slide surfaced with neutral red, and sealed with salvolin under a coverglass.

Small pieces from the area were fixed in methyl alcohol, Helly's solution, and 70 per cent ethyl alcohol. Paraffin sections were treated with Wright's stain and also with the Prussian Blue technique for free iron.

Small pieces of tissue were cut up, explanted into autoplasm or homoplasm on coverglasses surfaced with a film of neutral red, sealed over a slide cavity with salvoline and incubated. The explants were small and thin, and their cells visible. Cultures were stained with the Prussian Blue technique and with other methods.

Bovine tubercle bacilli were autoclaved, ground in a mortar, washed with normal saline and resterilized. 1 cc. of a thick suspension in physiological saline was injected subcutaneously into the flank of young white rats.

Tissue cultures were prepared in the usual manner from material removed at various intervals from the injected area. Incubated and unincubated cultures of the pieces of tissue were fixed in 10 per cent formalin, stained with carbol fuchsin for two hours, decolorized and

counterstained with hematoxylin for bacteria. Others were subjected to the Prussian Blue technique.

Human tubercle bacilli, H37, were similarly used.

Cultures of the buffy coat of rat's blood and of the rat's buffy coat with washed rabbit's erythrocytes and a small amount of rabbit plasma were made in auto- or homoplasma. The cultures were stained with the Prussian Blue technique.

Cultures of the rat's buffy coat in auto- or homoplasma with killed bovine and also with human, H37, tubercle bacilli were prepared by drawing 0.2 cc. of a suspension of the killed bacilli into a syringe containing the heparin into which the desired amount of blood was drawn from the heart. This was well mixed by shaking, after which the mixtures were centrifuged and cultures prepared in the usual way. After varying periods of growth some of the cultures were fixed with 10 per cent formalin, stained with carbol fuchsin, decolorized in acid alcohol, and counterstained with hematoxylin. Others were subjected to the Prussian Blue technique.

OBSERVATIONS

During the first 24 hours following the injection of the washed rabbit's erythrocytes, a few monocytes were demonstrable in the spreads and sections of the rat's tissue, but rarely after that time. These preparations were comparable to the picture found after 24 hours in the explant of rat's buffy coat (fig. 1). Many young macrophages were noted at 5 hours; and thereafter became more numerous. These increased slowly in size and in content of ingested erythrocytes and débris. In the process of digestion the erythrocytic envelope disappeared leaving behind intracellular crystals. These crystals were not tested chemically, but from their shape and color probably represented hemoglobin, hemin, or bilirubin. After 26 hours some of the iron had been set free and was demonstrated in the macrophages. This iron increased in amount in the older preparations (fig. 3).

The cultures were more favorable for observation. Those prepared from tissue removed 5 hours after the rat had been injected contained many monocytes, and but few macrophages. After these cultures had been incubated 24 hours, there were fewer monocytes, and the macrophages had increased in number and in size. Forty-eight hours

later, monocytes were rarely seen (fig. 2). The amount of ingested material (red blood cells and débris) in the cells had increased. Iron was not demonstrated in the macrophages in cultures before the fourth day, but thereafter it increased in amount.

The explants of the cultures prepared from tissue removed 24 hours and later after the injections into the rats contained no monocytes but revealed an increased number of macrophages, and those explanted later contained macrophages and some epithelioid cells. Following incubation there was an increase in the number and in the size of the macrophages and in the number of epithelioid cells. Intracellular iron first appeared in the 72-hour explants that were incubated for two days. The explants of tissue removed after several days contained increasing numbers of epithelioid cells (fig. 6).

The findings following the injection of bovine tubercle bacilli differed from the above in that monocytes were abundantly present in the tissue removed for explantation even as late as 72 hours after the injections. On incubation the number of monocytes decreased and the macrophages increased in number. There was also an increase in the size and in the amount of ingested material in these cells and, finally, in the amount of epithelioid cells. Explants of tissue taken five days and later showed no monocytes, but in these many large macrophages and an increasing number of epithelioid cells were found.

The results after the injection of human tubercle bacilli corresponded more clearly to those following the injection of red blood cells. The monocytes were relatively few in number in the tissue during the first day. After the first day, fewer were noted and the macrophages were found in increasing numbers. At 48 hours and later, epithelioid cells were also noted in increasing numbers. On incubation of cultures of these tissues the macrophages increased in number and size and finally epithelioid cells came to predominate (fig. 7).

Cultures of the buffy coat shortly after preparation contained many monocytes and few macrophages. Following incubation, however, there occurred a decrease in the number of typical monocytes with a corresponding increase in the number and in the size of the macrophages and, finally, the appearance of epithelioid cells. In cultures prepared from the buffy coat of rat's blood mixed with washed rabbit's

erythrocytes, the macrophages increased more rapidly in number and in size. The epithelioid cells also appeared earlier. Similar results were obtained in the cultures of blood mixed with tubercle bacilli.

At all stages of the cytological process tubercle bacilli could be found intracellularly (fig. 5), but no sufficient evidence of their digestion by monocyte, macrophage or epithelioid cell could be demonstrated during the length of time they were observed.

In cultures of the buffy coat to which no foreign material had been added mononuclears with large irregularly stained bodies were more numerous during the early hours of the culture than those with the typical rosette. In addition to these there were many cells without a rosette formation. As the culture became older, more and more cells became macrophages or epithelioid cells and lost the characteristics of the monocyte.

The monocytes were watched for varying periods of time during their increase in size and cellular content, and in two instances a monocyte having a typical rosette was followed continuously and observed to hypertrophy and to acquire large, stained bodies which lost the rosette formation and became irregularly placed around the nucleus so that in the end the cell no longer could be termed a monocyte, for it had become a typical macrophage.

While the monocytes were frequently observed to change into hypertrophied cells resembling one of the various types of macrophages of the tissue, none of the macrophages were seen to change into monocytes. If a hypertrophied cell used up its ingested material it became an epithelioid cell, not a monocyte. The hypertrophied cells divided by mitosis to form two large cells, not two monocytes.

DISCUSSION

In the comparison of the behavior of the monocyte and macrophage *in vivo* with similar cells *in vitro*, it was possible to determine that the monocytes become transformed into macrophages resembling those of the fixed tissue, such as the clasmatocyte and Kupffer cell, and into epithelioid cells, and also that macrophages change into epithelioid cells.

If, however, one were to classify the phagocytic cells without reference to their size, type of nucleus and cytoplasmic characteristics,

but simply on the staining peculiarities described by Sabin, Cunningham and Doan, it could then be stated that while freshly drawn blood contains cells with only few neutral red-staining bodies, films of blood studied in the warm box exhibit mononuclears with neutral red granules similar to those described by these investigators as characteristic of lymphocytes, of monocytes, and of macrophages. In these films one also finds varying gradations of staining such as has been shown by Maximow, the Lewises, and Bloom.

On the other hand, if these cells are followed for longer periods of time (24 hours to 4 days), as has been done in tissue cultures of hanging drops of blood (M. R. Lewis, and Lewis and Lewis) or in tissue cultures of the buffy coat (Carrel, Maximow, Bloom and Hetherington), it can be demonstrated that the monocytes of Sabin's monocytic type and of the so-called young "clasmatocyte" type, hypertrophy and change in nuclear and cytoplasmic characteristics, until they become typical macrophages and epithelioid cells such as are found *in vivo*. Maximow (16) and Bloom (16) claim that the lymphocytes also undergo transition into macrophages, but I was not able to show this in my preparations nor has it been corroborated by other investigators. In the types of preparations studied by most investigators up to the present time, the lymphocytes and the polymorphonuclear leucocytes die after a few days.

The transition of the monocytes into macrophages and epithelioid cells seemed to depend somewhat upon their environment as, for instance, in the subcutaneous tissue injected with bovine tubercle bacilli the monocytes persisted for 72 hours and cells in the cultures of tissue removed from the site of injection were further advanced in stages of transition than were those left *in vivo* for a comparable length of time. Also cultures of the buffy coat of blood mixed with foreign red blood cells exhibited macrophages containing ingested red blood cells (iron and crystals) earlier and more plentifully than did the cultures of the buffy coat of rat's blood alone. Cultures of buffy coat successfully separated from the layer of red blood cells tended to have more epithelioid cells than did those of the buffy coat with adherent red blood cells, and typical epithelioid cells appeared earlier in the cultures of buffy coat free from blood, while in those with red blood cells there were many characteristic macrophages.

Usually it is believed that the phagocytic cells common to the liver, spleen and bone marrow, where bile pigment is known to be manufactured, are responsible for its production. The work of Rich (28), and of Rich and Bumsted (27) has demonstrated a similarity between the macrophages and these cells in their ability to form bilirubin.

In my preparations the physiological behavior of the macrophages was found to be analogous to that of the mononuclear phagocytes of the sinusoids of the liver and spleen. The activity of these cells in breaking down the ingested red cells is well-known. The macrophages of the blood and connective tissues were likewise found to be the cells which digested the erythrocytes and freed the iron from the hemoglobin. At the time at which the intracellular iron was found, the neutral-red preparations contained large macrophages filled with neutral-red vacuoles and disintegrated material.

The transition of the monocyte during several days in tissue culture was characterized by a marked increase in the size of these cells so that, while the younger macrophages were only a few times larger than the monocyte (fig. 1), the macrophages in older cultures were sometimes as much as 10 times larger in area than the original monocyte, and the epithelioid cells (fig. 7) frequently attained an area as much as 20 or more times that of the monocyte. This agrees with figures shown by Lewis (20) and by Lewis and Lewis (19) in which some of the cells are as much as 20 or more times that of monocytes drawn from blood smears.

These comparative studies of *in vivo* and *in vitro* reactions of buffy coat and of connective tissue add further evidence that after certain periods of time and under the proper physiological influences the monocytes pre-existing in the blood stream hypertrophy and change into macrophages and epithelioid cells.

SUMMARY

The reactions of the mononuclear phagocytes of the subcutaneous tissues and of the blood towards washed rabbit's erythrocytes and killed bovine and human tubercle bacilli were studied in sections and supravitally in tissue cultures in a comparison of the behavior of these cells *in vivo* and *in vitro*.

Cells with the characteristics of monocytes phagocytized foreign

material, and changed into macrophages which increased in size and number. As the ingested material was reduced to a granular débris, gradually increasing numbers of these changed cells became transformed into epithelioid cells.

A monocyte was followed continuously and concomitantly with the increase in size and contents of the cell during its transition into a macrophage, the red-staining granules were observed to lose their rosette arrangement and to become irregularly placed around the nucleus.

Monocytes were observed to change into macrophages typical of various fixed tissue cells, but these changed cells were not seen to return to the earlier stage.

Division of the hypertrophied cells took place by mitosis and gave rise to two large cells, not to two monocytes.

From these experiments it was concluded that the various types of macrophages are transitions of the monocyte occurring under various physiological conditions.

The author wishes to express his appreciation to Mrs. W. H. Lewis and to Dr. W. H. Lewis for their help and criticism during the course of these experiments.

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EXPLANATION OF PLATE

FIG. 1. An untouched photograph of a tissue culture preparation from the rat's buffy coat stained supravitally with neutral red and taken after 24 hours of incubation. The variation in cell size is noticeable. The smaller cells contain the rosette characteristic of the monocyte, while the larger ones illustrate the early stage of transition from the monocyte to the clasmatocyte. Wratten K2 filter, ×950. 2 mm. Zeiss apochromatic, ×10 compensating ocular.

FIG. 2. An untouched photomicrograph of a 72 hour supra-vitally stained neutral red tissue culture explant from the subcutaneous tissues of a rat previously injected with rabbit's erythrocytes. The central cell with its typical rosette lying opposite the Hof of the nucleus is illustrative of the monocytic type rarely seen at this stage of development. The other cells are typically clasmatocytes. Wratten K2 filter, ×950. 2 mm. Zeiss apochromatic, ×10 compensating ocular.

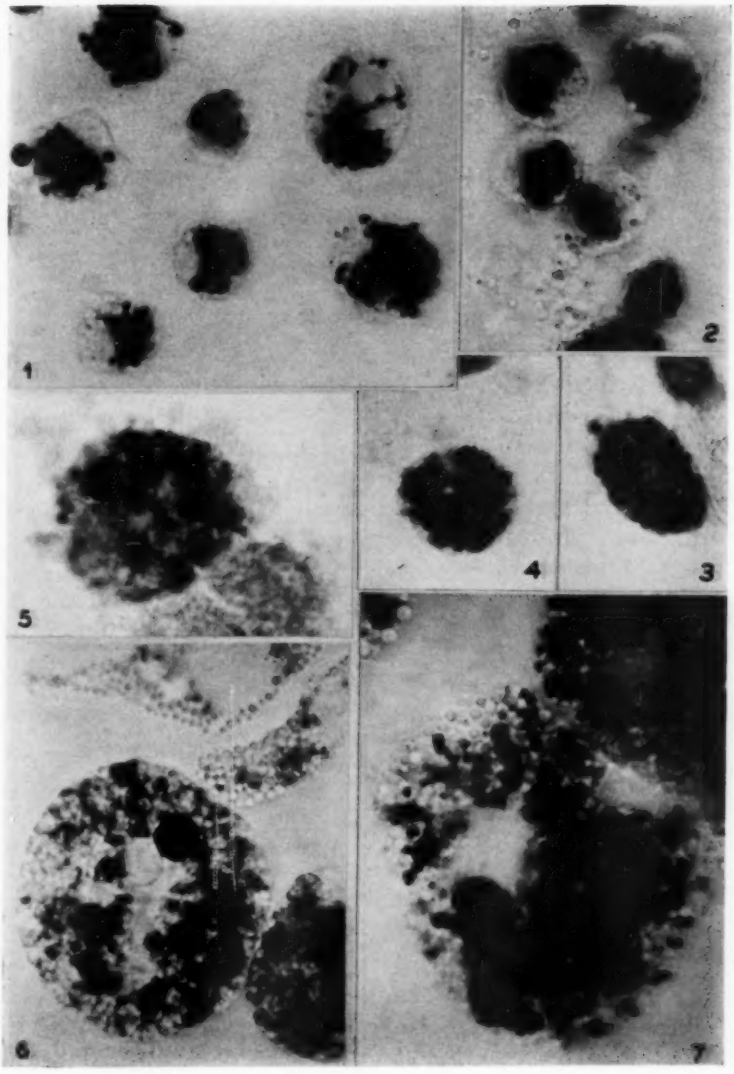
FIG. 3. An untouched photomicrograph of a 6 micra paraffin section of a clasmatocyte from the subcutaneous tissues of a rat injected 5 days previously with rabbit's erythrocytes; fixed in formalin-Zenker and stained by ther Prussian Blue technique. A large macrophage, typical of this period of maturation, is shown engorged with intracellular iron. Small amounts of free iron were found after 26 hours. Wratten B filter, ×1200. 4 mm. Zeiss apochromatic, ×15 compensating ocular.

FIG. 4. Tissue culture preparation from the rat's buffy coat incubated 6 days and stained with the Prussian Blue technique for iron. A large macrophage characteristic of this period of maturation is seen engorged with intracellular iron. Untouched photomicrograph, ×1200. Wratten B filter. 4 mm. Zeiss apochromatic, ×15 compensating ocular.

FIG. 5. An untouched photograph of a typical large epithelioid cell containing many clumps of phagocytized dead bovine bacilli, taken from a 14 day tissue culture preparation of the rat's buffy coat and stained with the acid-fast method. Wratten filter K2, ×1200. 4 mm. Zeiss apochromatic lens, ×15 compensating ocular.

FIG. 6. Supravitally stained neutral red tissue culture preparation of a 6 day explant from the rat's subcutaneous tissues previously injected with rabbit's erythrocytes and incubated for a 6 day period. A large epithelioid cell characteristic of this stage of development is seen in this untouched photomicrograph. Wratten filter K2, ×950. 2 mm. Zeiss apochromatic, × 10 compensating ocular.

FIG. 7. Large epithelioid cells supravitally stained with neutral red are seen in this untouched photomicrograph. This tissue culture preparation of a 4 day explant was taken from the rat's subcutaneous tissue previously injected with killed No. 37 tubercle bacilli and incubated for a period of 7 days. Wratten filter K2 ×1500. 2 mm. Zeiss apochromatic, ×7 compensating ocular.



SMITH OF BIOL.

IRON METABOLISM IN INFANCY

Relation to Nutritional Anaemia[1]

HUGH W. JOSEPHS

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

Submitted for publication June 9, 1934

There are three sources of iron for haemoglobin formation during an infant's growth: (*a*) The iron taken in with the food; (*b*) the iron present in the tissues at birth, especially the liver, in greater concentration than is needed for the immediate support of life (Bunge's stores); (*c*) the iron set free and deposited in the tissues during the fall in haemoglobin that takes place in the first two months of life. The purpose of this work was to determine, by means of balance experiments carried on continuously over a long period of time, to what extent retention of iron can cover the needs of the growing infant for haemoglobin formation and to what extent the organism must rely on iron already in the body at birth, either in the form of tissue deposits or in combination with haemoglobin.

METHODS

Six normal infants have been studied, four from two to three weeks of age through the third month, one from the sixth week to the sixth month, and one from the second week to the sixth month. The study in each case was virtually continuous. The individual periods lasted for five to six days out of each week, during which the babies were kept on a special metabolism bed which allowed the separate collection of urine and stools. The stools for an entire period were evaporated together on the steam bath to dryness, powdered, weighed dry and analyzed as described below. Since variations in the urinary excretion of iron were found to be of no significance, the urine was added to the stools and evaporated with them. The milk for each day's feeding

[1] Aided by the John Howland Memorial Fund.

was made up in the morning and a sample kept out for analysis. A careful record of the total amount of milk ingested and retained was kept and an aliquot of this was taken out of each day's sample. The aliquots for the period were combined and a portion taken for analysis. It was found to be unsafe to rely on the average iron content of milk for the calculation of iron intake, owing to the wide variation found in individual samples.

The method for iron analysis will be found at the end of the article.

In order to gain an approximate idea of the amount of iron set free from combination with haemoglobin during the period of falling haemoglobin, I have used the following calculations:—From the weight, the blood-volume is calculated on the assumption that the blood constitutes 7.6 per cent of the body weight. From the blood volume and the haemoglobin percentage the total haemoglobin can be calculated. The iron content of haemoglobin has been found to be 0.34 per cent (Butterfield's determination of the iron content of haemoglobin (1), confirmed by Peters' data on the relation of the iron content to oxygen capacity of blood (2)),[2] and from this figure the total iron combined with haemoglobin can be calculated.

Table 1 contains the data for weight and haemoglobin percentage, at birth or shortly after, and at the end of each month of observation. It is evident that the babies under study developed normally. One (5) was close to the borderline of prematurity, but gained satisfactorily; another gained more rapidly than the average, at least during the first four months. The others were within normal limits. One of the babies (2) had an upper respiratory infection during the fourth month which, however, was not of sufficient severity to influence the gain

[2] Two assumptions are of course implied in this calculation: (1) That the iron content of haemoglobin is constant; (2) that the haemoglobin per cent as determined by an acid haematin method bears a constant relation to the oxygen-carrying capacity of the blood. The second of these two assumptions has been shown to be correct for a large number of normal cases. Regarding the first, data soon to be published indicate that the iron of the whole blood can be entirely accounted for by the haemoglobin, as determined by the Newcomer method, at birth and after the eighth week of life. In this paper we are interested in the iron set free for haemoglobin by the end of the second month, and the fact that for a time this iron seems to be held in the blood before being deposited in the tissues does not for the present concern us.

either in weight or haemoglobin percentage. Otherwise the babies were free of infection.

In table 2 are the iron balances arranged in monthly periods. To obtain these figures the balances for individual periods are added together and adjusted to thirty days. The data for the individual periods have been omitted in order to save space, but are used in the construction of the charts. In columns parallel to those containing iron balances are figures for gain or loss in total haemoglobin iron, calculated as outlined above from the data of table 1.

From table 2 it may be seen that in five out of six cases there was a negative iron balance in the first two to three months of life, especially in the second month. The balance then became positive and remained so as long as the observations were carried on except in one case (J) in which there was infection. Although the period of negative balance corresponds in general to the period of falling haemoglobin, there was no relationship between the reduction of total haemoglobin and the negative iron balance. In fact the net amount of iron lost from the body was almost negligible even when, as in S and J, the amount of iron set free from combination with haemoglobin was considerable. From the end of the second month the positive balance corresponded to a period of rising haemoglobin, but in this period also there is no clear relation between the body's gain in iron and the increase in total haemoglobin iron, the positive balance covering only a very small percentage of the total needs.

The lack of any close relationship between excretion and fall in haemoglobin leads to the conclusion that other factors are of importance. The charts illustrate one or two of these. As may be seen, the most marked negative balance was obtained from a child on breast milk (chart 1, T.W.). It is not clear what factor was responsible here, but it is not unlikely that it was the low iron content of the breast milk as compared with the other types of feeding used. The effect of breast milk is also seen in chart 2 (case 1, S). In the case of T the use of barley flour increased the iron content to a figure higher than in the other babies, and the iron balance was positive throughout (chart 3, T.). A second factor that influences the excretion of iron is the presence of infection as shown in chart 4. This was a very mild infection manifested by a rise in temperature to about 100° for a few

TABLE 1

		WITHIN FIRST WEEK		END OF FIRST MONTH		END OF SECOND MONTH		END OF THIRD MONTH		END OF FOURTH MONTH		END OF FIFTH MONTH		END OF SIXTH MONTH	
		Weight	Haemoglobin	Weight	Haemoglobin	Weight	Haemoglobin	Weight	Haemoglobin	Weight	Haemoglobin	Weight	Haemoglobin	Weight	Haemoglobin
		kg.	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*	*kg.*	*gm. per 100 cc.*
1	S.	2.5	17.2			3.3	10.3	4.4	10.4	5.1	10.9	5.5	11.0	5.8	11.2
2	J.	3.7	20.6	4.1	16.0	4.9	10.7	5.5	11.0	6.1	12.9	6.9	12.0	7.2	13.4
3	Ko.	3.5		4.6		5.0		5.9		6.4					
4	T. W.	3.6	15.2	3.8		4.3		4.9		5.7					
5	Ke.	3.6	17.0	4.7	10.9	5.8	10.7	6.5	11.7	6.8	13.1				
6	T.	3.4	15.3	4.0	12.2	4.7	9.7	5.1	12.0						

TABLE 2

			BIRTH TO 1 MONTH		1 TO 2 MONTHS		2 TO 3 MONTHS		3 TO 4 MONTHS		4 TO 5 MONTHS		5 TO 6 MONTHS	
			Iron balance	Increase or decrease in iron combined with haemoglobin	Iron balance	Increase or decrease in iron combined with haemoglobin	Iron balance	Increase or decrease in iron combined with haemoglobin	Iron balance	Increase or decrease in iron combined with haemoglobin	Iron balance	Increase or decrease in iron combined with haemoglobin	Iron balance	Increase or decrease in iron combined with haemoglobin
			mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	S.	Whole milk formula			−3.8	−23†	+4.5	+28	+5.2	+29	+6.0	+12	+4.5	+11
2	J.	Lactic acid milk formula	−2.05	−27	−1.4	−34	+7.3	+20	−1.7‡	+48	+6.7	+11	+5.2	+33
3	Ko.	Whole milk formula	+.21*		−1.2		−1.5							
4	T. W.	Breast milk	+1.3*		−7.9									
5	Ke.	Whole milk formula		−31	−3.9	+28	+6.7	+37	+5.0	+33				
6	T.	Whole milk formula with barley flour	+10.4	−10	+2.0	−7	+5.2	+41						

* Two weeks. ‡ Infection. † Two months.

days, and a coryza lasting about two weeks. The effect of a more severe infection with somewhat greater loss of iron is illustrated in

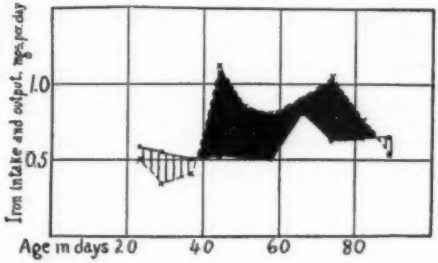


CHART 1. IRON INTAKE AND OUTPUT IN CASE 4 (T. W.)

Note: Fed on breast milk throughout the period of observation. Iron intake was somewhat lower than in the other cases and the negative balance was more pronounced.

In the charts the iron intake is designated by the continuous line, the output by the broken line.

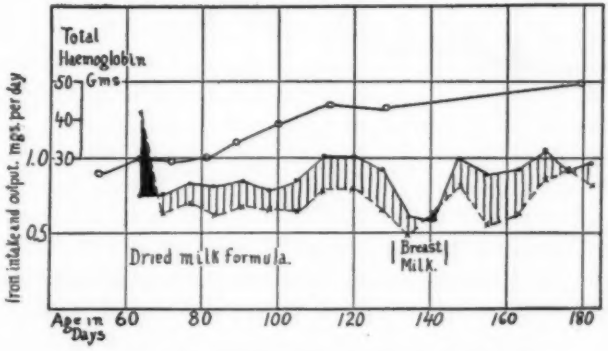


CHART 2. IRON INTAKE AND OUTPUT IN CASE 1 (S.)

Note: Observation began at the end of the second month. Breast milk feeding was accompanied by a marked reduction in iron intake.

chart 5. This child, eight months of age, developed otitis media just after a study of iron balance had been begun, and a preliminary control period had been completed. A third infant was studied for a short

* Two weeks. ‡ Infection. † Two months.

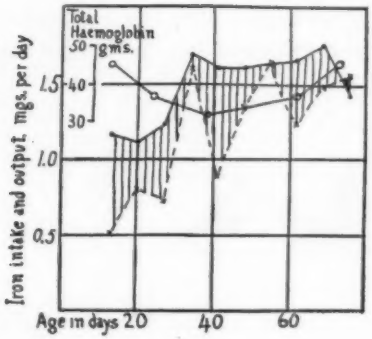


CHART 3

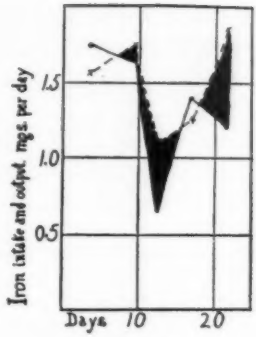


CHART 5

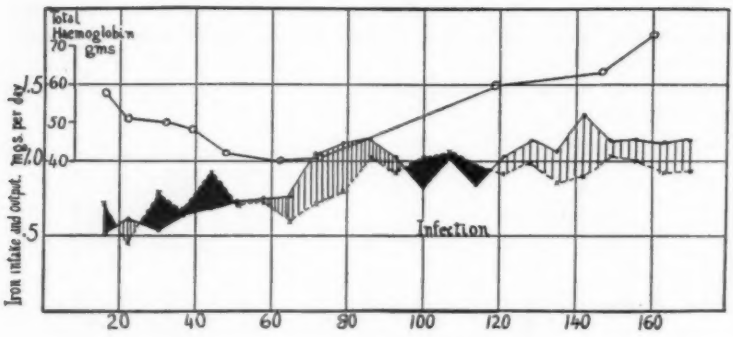


CHART 4

CHART 3. IRON INTAKE AND OUTPUT IN CASE 6 (T.)

Note: The milk in this case was supplemented with barley flour. The iron intake was higher than in the other cases and there was no negative balance.

CHART 4. IRON INTAKE AND OUTPUT, IN CASE 2 (J.)

Note: The child was fed a formula made from dried lactic acid milk. The iron intake was moderate. Note the negative balance during the second month of life and later during an infection.

CHART 5. IRON INTAKE AND OUTPUT IN AN INFANT OF 8 MONTHS

Note: This child had an infection (otitis media), beginning on about the seventh day of observation.

period during the course of a pneumonia, and a daily loss of about 1.5 milligrams of iron was found. Compared with the total iron of the body this seems like a small amount. We have, however, no information on how long such a loss might be continued in the case of a prolonged infection or how great the daily loss might be in a pneumonia more severe than in the case studied.

From table 2 it may be seen that only a small proportion of the iron utilized for haemoglobin formation from the second to the sixth month is derived from the retention of iron during this period. It is, therefore, evident that the major portion of iron entering the haemoglobin molecule must come from iron deposits already in the body by the end of the second month. Table 3 is a balance sheet for the cases under consideration in this paper, to determine to what extent iron present at birth was drawn on to supply iron for haemoglobin at the end of the third, fourth and sixth months of life. The procedure was as follows: From the fall in haemoglobin could be calculated the amount of iron set free from its combination with haemoglobin. The net increase in tissue deposits during the first two months was found by subtracting the negative balance from the iron derived from haemoglobin. These are the figures in the fifth column. This iron, in addition to that represented by the positive balance beginning with the third month, is considered as available for new haemoglobin formation. Thus in S. at the end of the second month about 19 milligrams were deposited in the tissues during the fall in haemoglobin. During the third month about 5 milligrams were retained, bringing the available iron from these two sources to 24 milligrams. The gain in haemoglobin the third month was, however, 28 milligrams, so that 4 milligrams were required to be furnished by deposits that must have been present at birth or have been laid down shortly after birth before the first haemoglobin determination was made. Similarly, at the end of the fourth month 10 milligrams had been retained, so that 29 milligrams were available from the two sources exclusive of "stores" at birth; 57 milligrams were needed for haemoglobin, so that 28 milligrams were required from stores already in the body at the time the study was begun. The same method of calculation has been applied to all the available material.

From a study of this table certain facts may be gleaned, in spite

of the marked individual variability. At the end of the third month in three out of the four cases the stores with which the child was born were virtually unused, and in one of these the use of the "stores" did not begin until the end of the fourth month. In the fourth case (Ke.), owing to a particularly rapid gain in weight, the necessity for "stores" at birth became apparent before the end of the third month. By the end of the sixth month in two cases (S. and J.) the amount required to be made up from "stores" that must have been present at birth was about 40 milligrams, while in the case of Ke by the end of the fourth month this amount was about 60 milligrams. There are in the literature determinations of iron in the livers and spleens of newborn babies dying from various causes (3, 4). The figures vary considerably, but 40 to 60 milligrams would be a fair average for the total iron in the blood-free liver, with a maximum up to 80 to 100 milligrams and a minimum of 10 to 15 milligrams. The iron in the spleen was much smaller in amount, but increased after birth. The iron of the liver may be accepted as representing the available stores in Bunge's sense, and it has been shown that it tends to reach its lowest point about the third or fourth month of life (4). There is, of course, other iron in the body especially in the muscles, but such iron is probably not available for haemoglobin formation.[3]

The figures in the last column of table 3, representing that portion of the iron requirements not derived from the food or the destruction of haemoglobin, signify the minimum amount of iron that must have been present at birth and available for haemoglobin formation. The average content of iron in the liver at birth approximates these figures. The significance of this fact is apparent, for it means that under average conditions there is sufficient iron at birth to maintain a normal haemoglobin to about the fourth to the sixth month of life, a conclusion that corresponds very well with clinical experience.

Thus far we have dealt with factors which concern the maintenance of normal haemoglobin up to six months of age. The development of an iron-deficiency anaemia on a milk diet after exhaustion of available iron deposits is another phase of the problem which can be dealt with

[3] In the rat it was found that somewhat more than half the iron of the body not combined with haemoglobin was present in the muscles, but that this iron was not available for haemoglobin formation (unpublished data).

TABLE 3

		HAEMOGLOBIN-IRON AT BIRTH	FALL IN HAEMOGLOBIN-IRON DURING FIRST TWO MONTHS	IRON BALANCE DURING FIRST TWO MONTHS	IRON DEPOSITED IN TISSUES DURING FIRST TWO MONTHS	IRON BALANCE			TOTAL IRON AVAILABLE EXCLUSIVE OF "STORES" AT BIRTH			INCREASE IN HAEMOGLOBIN-IRON			BALANCE OF IRON REQUIRED FROM "STORES" PRESENT AT BIRTH		
						2 to 3 months	2 to 4 months	2 to 6 months	2 to 3 months	2 to 4 months	2 to 6 months	2 to 3 months	2 to 4 months	2 to 6 months	2 to 3 months	2 to 4 months	2 to 6 months
1	S.	111	23	−4	19	+5	+10	+20	24	29	39	28	57	80	4	28	41
2	J.	197	61	−4	57	+7	+6	+18	64	63	75	20	68	112	−44	5	37
5	Ke.	163	3	−4	−1	+7	+12		6	11		37	70		31	59	
6	T.	136	17	+12	29	+5			34			41			7		

TABLE 4

	AGE	IRON RETENTION 6 MG. PER MONTH	WEIGHT	TOTAL HAEMOGLOBIN-IRON	TOTAL HAEMOGLOBIN	BLOOD VOLUME WEIGHT × 76	HAEMOGLOBIN
	months		*kg.*	*mg.*			*gm. per 100 cc.*
Exhaustion of "iron stores" at 6 months	6	0	6.5	200	59	495	11.9
	12	36	9.5	236	69.5	720	9.6
	18	72	11	272	80	835	9.6
Exhaustion of "iron stores" at 4 months	4	0	5.5	156	46	420	11
	6	12	6.5	168	49.5	495	10
	12	48	9.5	204	60	720	8.3
	18	84	11	240	70.5	835	8.5
Exhaustion of "iron stores" in 3 months	3	0	5.0	130	38	380	10
	6	18	6.5	148	43.5	495	8.8
	12	54	9.5	184	54	720	7.5
	18	90	11	220	65	835	7.8

here only in a general way. When the reserve iron is exhausted, the fall in haemoglobin will depend on the iron available from the food and the rapidity of gain in weight. For the present we may leave out of account pathological conditions, and consider how far iron deficiency alone can be used to explain the development of nutritional anaemia.

In table 4 I have attempted to gain some idea of the extent of the reduction of haemoglobin due to growth, supposing the iron stores to be exhausted at, respectively, three months, four months and six months. As shown above, it would probably be quite exceptional to encounter exhaustion of iron reserves at three months in a normal child, while at six months presumably the great majority of infants must begin to depend on the iron of the food for the necessary additions of iron to the body store. From the figures presented earlier, the retention of iron from the milk is fairly constant from the second to the sixth month and it is fair to assume that in the normal infant this retention will be continued at the same level beyond the sixth month. In the table this retention is assumed to be 6 milligrams a month (see table 2). The weights are those of an average child. As an example of the manner in which the table was constructed, let us assume a child whose iron deposits are exhausted at four months of age. He would weigh about 5.5 kilograms and his haemoglobin would be about 11 grams per 100 cc. He would have about 46 grams of total circulating haemoglobin with an iron content of 156 milligrams. At six months 12 milligrams of iron would be added from the food, bringing the available haemoglobin iron to 168 milligrams, corresponding to 49.5 grams of haemoglobin. His weight would now be about 6.5 kilograms and his blood volume 495 cc. His haemoglobin concentration would therefore have fallen to 10 grams per 100 cc. Continuing this calculation throughout the table we see that we cannot by this means account for any great degree of anaemia (7 grams per 100 cc. corresponds to about 50 per cent haemoglobin on the usual clinical scale) and that after the twelfth month the probable intake of iron essentially covers the needs for new haemoglobin formation.

In making these calculations I have not taken into account the iron of the tissues, present in the nucleus and unavailable for haemoglobin. The only figures that bear on this subject are those in a pre-

vious study on rats (5) in which it was found that this unavailable tissue iron amounted to about 5 milligrams per kilogram of animal. If this figure can be applied to human tissue, it would mean that not more than 30 milligrams of iron would be required for the function iron of the tissues during growth from the third to the eighteenth month. It is more likely that this figure is lower in the human, than that it is higher, so that 25 to 30 milligrams probably represent the maximum requirement for tissue iron. Taking this iron requirement into consideration would lower the haemoglobin percentage at six, twelve and eighteen months, by a small amount. In the case of the child in the first section whose stores are assumed to be exhausted at three months, at twelve months the haemoglobin concentration would be 6.5 instead of 7.5 and at eighteen months the same.

From these calculations we may conclude that it is not possible to account for an iron-deficiency anaemia in the average child on the basis of a relative absence of stores at birth and a normally rapid growth. We must look for something more, that actually diminishes the total amount of iron in the body or increases the relative rate of growth. Prematurity does both these things, for according to Lichtenstein's figures (6), the negative iron balance is both greater and more prolonged than I have found in the normal children reported in this paper, so that iron deposits would be exhausted rather earlier, probably about the third month in most cases or even before this time. In addition, premature babies gain relatively more rapidly than full-term babies during the first year, and the haemoglobin percentage is usually considerably lower than that of full-term babies at the end of the third month. All these factors combine to make iron-deficiency anaemia much more likely in the premature than in the full-term baby, without having recourse to the absence of stores at birth for an explanation. It is not altogether correct to say that premature babies are born without stores. In relation to weight they are nearly if not quite as well supplied with iron as the full-term baby. It is because of the relatively more rapid gain that the iron deposits are insufficient. A second factor in diminishing the iron content of the body is infection, as indicated above. From the figures in this paper it is impossible to determine how important infection might be. 4.5 milligrams in three days, which is the highest figure obtained in a case of pneumonia, is

after all not a very great amount when compared with the total iron of the body. Further study along this line is desirable, to determine whether the so-called iron-deficiency anaemia after infection is due to a net loss of available iron from the body or to some factor rendering the body incapable of using the iron deposited in the tissues. Surprisingly little has been done to study the metabolism of iron. In recent years only four papers have appeared: One by Wallgren (7), consisting of a number of fairly widely spaced periods of observation, merely shows that the balance is generally positive and that the few periods with negative balance occurred in the first three months of life. In a paper, by Beck (8), dealing with iron balance in the premature, it is shown that extra iron given by mouth is not stored in the body for future use but is rapidly excreted after the administration of iron is stopped. Snelling (9) finds, in disagreement with Lichtenstein, that, as a rule, there is in premature babies a positive iron balance. Schlutz (10) was concerned with the value of vegetables as a source of iron. Until we know more of the factors influencing iron metabolism than we do now, we cannot hope to understand the mechanism of iron-deficiency anaemia.

SUMMARY

The iron metabolism of six normal infants was studied to determine: (*a*) to what extent an infant conserves the iron stores with which he was born, and (*b*) to what extent these stores are required to cover the needs for haemoglobin formation after the second month of life. It was found that in the first two months of life there tended to be a negative iron balance which, however, did not cause a loss of more than a few milligrams during this period. From the end of the second month the balance was positive. The gain in iron was approximately constant and averaged about 6 milligrams a month. The effect of infection was to cause a negative balance, but the importance to be attached to such a negative balance could not be determined, in view of the limited number of observations. From calculations based on the data from the six cases studied, it was concluded that the "birth-stores" are required for haemoglobin formation only rarely before the third month of life. From a comparison of the amount of iron required for haemoglobin formation from the second to the sixth month, with that

found by various investigators to be present at birth, it was concluded that the stores were probably sufficient to last up to the end of the sixth month under normal conditions. A further calculation indicated that an exclusive milk diet cannot cause any great degree of anaemia on the basis of its low iron content alone, but that other factors causing a disturbance in iron metabolism must be present.

METHOD FOR IRON DETERMINATION

The collection of the material and its preparation have been considered earlier, down to the point of weighing the sample. The weighed sample of dried stool, usually about 2 grams, was placed in a platinum dish and ashed dry in an electric furnace, care being taken to avoid letting it smoke. The milk sample—25 cc. of the mixed aliquots of each day's feeding—was placed likewise in a platinum dish, evaporated on a steam bath and ashed slowly in the electric furnace. On completion of the ashing, the ash was dissolved in 15 cc. 1 N HCl. In the case of the stools the amount of iron present necessitated taking only a portion of the solution of ash for the determination. The solution, therefore, was transferred to a 50 cc. volumetric flask, and 10 cc. of the diluted solution were taken for the determination. In the case of milk all the ash solution was used, so that it was transferred immediately to a small flask to be kept until a series of iron determinations was to be made.

The determination of iron was done as follows. To each flask was added three drops of concentrated nitric acid and the flask placed on a steam bath for twenty minutes or more. (The procedure of heating on a steam bath is absolutely necessary just before a determination is to be made, even though the oxidation of the iron might have been accomplished previously. If it is not done low values will be obtained.)

The flask was then cooled under the tap and transferred to one of two 50 cc. glass-stoppered cylinders. Into the other was placed 5 cc. N HCl and about 10 cc. of distilled water. Five cubic centimeters of 20 per cent sodium thiocyanate were added to each cylinder, then 10 cc. amyl alcohol. To the second cylinder was added a standard iron solution[4] containing 0.02 milligrams of iron per cubic centimeter made up in N HCl, until the colors in the alcohol layer nearly matched. Water was added to bring the water layer in each cylinder to approximately the same point. The amyl alcohol layers were pipetted off into colorimeter cups and compared.

A small error is present because of the different amounts of HCl in the two solutions. In the determinations reported in this paper the error amounted to less than 1 per cent and has been disregarded.

[4] The $FeCl_3$ solution used as standard was made up from iron wire (for analysis) and the stock solution contained 10 grams of Fe per litre. The dilute iron solution used as standard in the determination was made up fresh from the stock solution each time a series of determinations was to be carried out.

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ON THE LOCOMOTION OF THE POLYMORPHONUCLEAR NEUTROPHILES OF THE RAT IN AUTOPLASMA CULTURES

WARREN H. LEWIS

From the Department of Embryology, Carnegie Institution of Washington and the Johns Hopkins Medical School, Baltimore, Md.

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This study is based on a motion picture of rat polymorphonuclear neutrophilic leucocytes in a small hanging drop culture of small pieces of the buffy coat in autoplasma. The buffy coat was obtained by centrifuging whole blood, drawing off the supernatant plasma, then lifting out the buffy coat layer into a dish of Locke's solution and cutting it up into small pieces for explantation into a small drop of the plasma.

The neutrophiles are the first cells to migrate out into the plasma clot and they begin to do this within a few minutes. The migration continues vigorously for about 24 hours. After that many of these cells lose their attachment to the coverglass, sink to the bottom of the clot and usually assume a spherical form. The motion picture was made at 9 to 10 hours' incubation, at 38° to 39°C., at 24 frames per minute, and at an initial magnification on the film of 165 diameters. When projected at the normal rate of 960 frames per minute, motion is increased 40 times. Only a general notion of the behavior of the cells can be determined at this speed. The method for the detailed study of the films at 2000 diameters has been described (Lewis, 2, 3). The frames are projected one at a time on paper and outlines of the cells drawn. Thus changes in form and in position at $2\frac{1}{2}$ second intervals or any multiple thereof can be recorded. In this manner the mode of migration and the rate of migration were determined for a number of different leucocytes.

THE MODE OF LOCOMOTION

The paths of the leucocytes as they migrate on the coverglass across the field are extremely variable in direction. They pursue zig-zag

courses; some continue in the same general direction (Fig. 1); others circle around in varying degrees. The observations are confined to cells migrating on the coverglass.

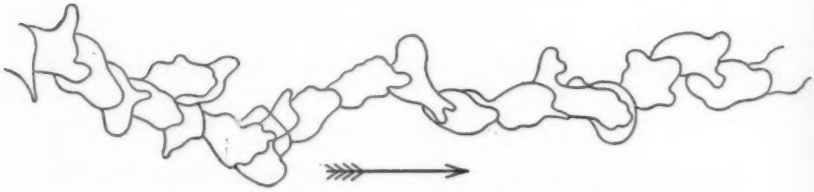

FIG. 1. PATH OF CELL 20 PLOTTED AT 20 SECOND INTERVALS FROM FILM 40. × 1000 DIA.

The elongated migrating neutrophiles are continuously changing in length and in width. An individual leucocyte may change from 12 to 23 microns in length and from 10 to 6 microns in width. The succession of changes in form occur at irregular intervals of 20 to 50 seconds and correspond to the frequency of, to the intensity of, and to the position of the constriction rings which tend to compress and elongate it. The migrating neutrophiles tend to keep the same anterior and posterior ends over a considerable period of time (figs. 1 and 2). Three regions can usually be recognized, namely, pseudopodal, body and tail. Each part varies in distinctness and in length from time to time. The pseudopodal part varies from 4 to 12, the body from 6 to 12 and the tail from 2 to 5 microns in length. With an average migrating length of 18 microns, the pseudopodal region is about 7, the body about 8 and the tail about 3 microns long. The thin irregular pseudopodal region is the most variable in form and size and in the extent to which it is spread out on the coverglass. The direction in which the pseudopodia are thrust out from time to time determines the direction of migration and the variability in the path. The pseudopodal end appears to be more adherent to the coverglass than the body. The tail does not seem to be adherent. Both the body and tail frequently show sudden slight shifts in position due apparently to changes in the pseudopodal region. The body of the neutrophile is fairly smooth and regular and only occasionally gives out a pseudopod. It is usually larger at the pseudopodal end and

tapers off to the tail. One can frequently observe constriction rings at both anterior and posterior ends which tend to separate it from the pseudopodal part and from the tail. The tail varies considerably in prominence depending upon the time relation to the constriction rings.

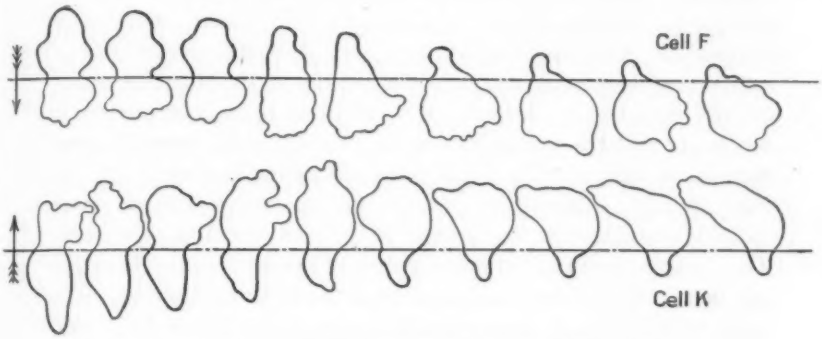

FIG. 2. NEUTROPHILES K AND F AT 2½ SECOND INTERVALS

The lines represent a constant distance from the edge of the field; the constriction rings through which the lines pass remain fixed in space while the cells move forward.

The most significant indicators of the mode of migration are the constriction or contraction rings which show with varying degrees of distinctness. They first appear at the base of the pseudopod between it and the body (fig. 2) and as the cell moves forward they remain fixed in position as regards objects outside the cell. Thus the neutrophile appears to flow through this constricted region until the posterior end arrives, and then the constriction ring contracts still more to emphasize the tail part. These rings succeed one another at intervals of about 20 to 50 seconds. They frequently disappear from view, they may be more marked on one side than the other, they are usually less obvious over the body of the cell than at the junction of body and pseudopod or body and tail. The neutrophiles shown in figure 2 were selected because the constriction rings are more obvious than in most of the others that were drawn.

The general structure of the neutrophile appears to be much like that of the lymphocyte. The cytoplasm of the pseudopodal end is

fluid, its granules are frequently in motion and can sometimes be seen to flow forward. The surface of the body and especially tail seems to be rather firm and gelated.

These observations indicate that the mode of locomotion of the neutrophile is essentially the same as for the lymphocyte (Lewis, 2, 3) and for the amoeba (Mast). In an early note on the migration of neutrophilic leucocytes (Lewis, 1), I described the constriction or contraction rings as passing from the anterior to the posterior end of the cell like a wave. With the introduction of the motion pictures it was found that the constriction rings remain fixed as regards objects outside the cell, as with the migrating lymphocytes, while the cell flows forward. The constriction rings of the neutrophile are more variable than those of the lymphocyte but are essentially the same. The diagram presented for the migration of the lymphocyte (Lewis, 3) can be applied without much modification to the neutrophile. The mode of migration of the neutrophile is not so obvious and probably would not have been recognized without the previous work on lymphocyte and amoeba.

It will be noted in figure 2 that both the anterior end and tail end move forward while the constriction ring remains fixed, and that while the body of the cell is built up in front of the constriction ring, it is reduced behind it.

The indications are that the body consists of a central plasmasol and an outer relatively thick plasmagel layer, which extends from the pseudopod to the tail; that the latter consists almost and at times entirely of plasmagel; that the pseudopod consists of an outer relatively thin plasmagel layer, surrounding a relatively abundant plasmasol. From the behavior of the constriction ring, the tapering shape of the posterior part of the body and the contracted tail it seems reasonable to assume that the body, especially that part posterior to the ring, is in a state of continuous contraction and that this forces the central fluid plasmasol forward into the pseudopod pushing that forward. Parallel with this there is probably a continuous change of the inner zone of the posterior part of the plasmagel layer of the body into plasmasol and a continuous gelation of this at the surface in front of each constriction ring to form a continuously advancing shell of plasmagel.

RATE OF LOCOMOTION

Only a few determinations of the rate of locomotion of neutrophiles *in vitro* have been made. Jolly (1913) found human leucocytes migrated 15.5 microns per minute at 37°C. and rabbit leucocytes 16.3 microns at 38°C. Comandon (1919) found the rate of human leucocytes at 35°C. to be 25.2 microns per minute. McCutcheon (1926) observed 100 human leucocytes, ten from each of ten different experiments, for five minutes each in thin layers of whole blood at 37°C. and found the average rate to be 34.1 microns per minute. If allowance be made for differences in temperature, McCutcheon points out that the figures given by Comandon agree quite closely with his. The general average rate of the 20 neutrophiles of the rat reported in this paper was only 19.4 microns per minute. They were in hanging drop cultures of the buffy coat at 38° to 39°C, and at 9½ hours *in vitro*.

The maximum rates for short periods of 20 seconds varied from 26.1 to 55.5 microns per minute, the minimum rates for similar short periods varied from 2.7 to 12.0 microns per minute. The average rates for the individual leucocytes during the entire period of observation varied from 13.6 to 24.9 microns per minute. The general average rate for the 20 leucocytes was 19.4 microns per minute. The rate of locomotion of every leucocyte varied from moment to moment and was never uniform. The periods of observation, that is, the length of time they were in the focal field of the motion picture varied from 3⅓ to 19 minutes. Since the motion picture was taken at the rate of one frame every 2½ seconds, it was possible to measure the rate for this period or any multiple of it. We used every eighth frame for this purpose, thus charting the position of the cells at 20 second intervals. For example, the distances traversed in each successive 20 second interval of leucocyte no. 27 during a period of 9 minutes and 20 seconds were as follows: 7.2, 1.9, 4.4, 9.8, 18.5, 6.3, 6.0, 10.0, 5.1, 9.6, 3.7, 9.8, 8.7, 3.1, 8.2, 3.2, 8.6, 7.8, 6.5, 6.0, 3.6, 4.1, 4.0, 4.0, 6.5, 4.7, 2.2 and 6.8 microns. The greatest distance was 18.5 microns in 20 seconds; this is at the rate of 55.5 microns per minute. Cell no. 20 (see figure 1), which had an average rate of 24.5 microns per minute, traversed in each successive 20 second interval during a period of 6⅔ minutes the following distances: 9.0, 6.2, 4.4, 9.5, 7.5, 4.2, 12.0,

10.4, 6.2, 13.4, 7.8, 10.9, 7.8, 6.4, 10.6, 9.6, 4.0, 14.5, 7.3, and 7.4 microns.

The average rate of locomotion is thus considerably less than that obtained by McCutcheon but the conditions in the two cases were quite different. We are not in a position to determine which approaches more nearly the rate in the body or to assume that human neutrophiles move more rapidly than those of the rat without comparison under similar conditions. It is interesting to note that rat lymphocytes migrate under similar conditions a trifle faster than neutrophiles.

SUMMARY

The mode of migration of the rat neutrophile is similar to that of the lymphocyte and the amoeba. The elongated migrating neutrophiles tend to keep the same anterior or pseudopodal and posterior or tail ends. A series of constriction rings form from time to time at the base of the pseudopod and remain fixed as regards objects outside the cell, while the latter appears to flow through them by a process of continuous solation of the inner surface of the outer plasmagel layer of the body of the cell and a continuous gelation to form an advancing outer plasmagel layer in front of each newly formed constriction ring. The central plasmasol appears to be forced forward into the advancing pseudopod by the continuous contraction of the body of the cell.

The rate of locomotion of 20 neutrophiles averaged 19.4 microns per minute.

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A CRITICAL REVIEW OF 170 CASES OF URTICARIA AND ANGIONEUROTIC OEDEMA FOLLOWED FOR A PERIOD OF FROM TWO TO TEN YEARS[1]

ARTHUR I. FINK, M.D. AND LESLIE N. GAY, M.D.

From the Protein Clinic of the Medical Department of The Johns Hopkins Hospital

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Of the many allergic manifestations, those that involve the skin are most confusing and the mechanism of their production is the least understood. Sir Thomas Lewis (1), in his book "The blood vessels of the human skin and their responses," details experiments on the physiologic reactions of the skin and the allied erythemata. His researches suggest that urticaria is the widespread flare resulting from the opening up of strong arterioles, which is due to a local reflex mediated through the terminal branches of the sensory nerves and initiated by the release of his so-called H-substance in the skin. It is assumed that the area covered by the wheal marks out the area of the skin in which this substance has been released; and that it is released in one of two ways; namely, either by direct injury to the cutaneous cells, as by external trauma, or by circulating or entrapped poisons. He elaborates the latter assumption by emphasizing that the production of various forms of skin eruption may depend upon the distribution of such poison, its potency, or the rapidity with which it injures living cells and liberates H-substance therefrom, the time that the poison lies in active form in the skin, its diffusibility, and the reactivity of the vessels and nerve endings concerned in the release of the H-substance. It is this group of factors, which in his opinion are so interrelated as to modify the reactions as we see them.

In urticaria where there is more or less widespread erythema surrounding a central wheal, he believes that there is an almost instantaneous poisoning of the cutaneous cells, whereby H-substance is released quickly and in large quantities; after which the arteriolar

[1] This investigation was made possible by the kindness of Mr. H. A. B. Dunning, Baltimore, Maryland.

dilatation helps to explain the quick outpouring of fluid to form a well defined and tense wheal.

The question as to whether or not this H-substance is histamine is by no means settled. Because histamine itself has not yet been chemically separated from skin extracts, Lewis cautiously refers to the active constituent as the H-substance, held in an inert form in the epidermal cells until liberated by injury. Sir Henry H. Dale (2), in his recent lectures in Baltimore, states that he is in accord with the experimental evidence that histamine is already in the cell, and that the union of antigen and antibody does not cause its formation by some newly initiated fermentative action, for a physical disturbance need only be supplied for its release. He also refers to a recently identified tissue constituent, choline, which may play a part in the production of local vascular reactions, especially in the larger arterioles, and he emphasizes the fact that what is known of the reaction between this H-substance and the active principle of choline, namely acetylcholin, points to a reaction immediately about the point at which they are liberated in the tissues.

The investigations of Walzer (3) have been most instructive as to the production of wheals by the absorption of undigested protein from the gastro-intestinal tract. His work needs no review.

The literature that has appeared during recent years is indeed confusing in so far as the etiology of urticaria is concerned. Many investigators have depended entirely for diagnosis of the causative factor on the interpretation of skin reactions or elimination diets. Conclusions have been reached before sufficient time has elapsed to establish the etiology. In general, results of treatment have been disappointing. Hopkins and Kesten (4), for example, report that they were unable to reach any definite conclusion as to the cause of the urticaria in 193 of 214 cases. To quote them; "Of 139 patients, 44 showed sufficient improvement on elimination diet to suggest that food was a causative factor. Twenty had foci of infection which were not cleared up under treatment, so that the relationship of such foci to the eruption remained doubtful." This summary of the merits of treatment, on the face of it, might be considered quite discouraging.

Our group of 170 cases has been under observation for from two to ten years. Each patient has been thoroughly studied as a diagnostic

problem from all angles; and an accurate follow up report has been made within the past two months. The analysis of the accumulated data is instructive and leads us to feel somewhat more optimistic than heretofore, in determining both the cause and treatment of urticaria and angioneurotic oedema. No attempt is made to present a complicated classification of the disease; our cases fall into the following definite simple groups:

I. Urticaria associated with foci of infection.

II. Urticaria, including those patients sensitive by the intradermal test to specific foods; sensitive by ingestion to specific foods and drugs; sensitive to externally applied physical agents (heat and cold, wool and silk); and sensitive to skin parasites (scabies).

III Urticaria dependent on and produced by psychogenic disturbances.

IV. Urticaria due to disturbances of the endocrine glands; disturbances of ovarian or thyroid function.

V. Urticaria of undetermined cause.

With this simple classification it is possible to analyze clearly the entire group of our patients and to present specific figures of the result of treatment whether it be by elimination of the food or of foci of infection; to point out the fundamental factor in the endocrine syndrome, and the psychic disturbances which are basically responsible, respectively, for the recurring urticarial explosions in these groups. We have studied every recorded variation from the normal in an effort to determine whether any such abnormality was peculiar to each group of cases in this series.

The group in general (*table 1*). Of these 170 patients, 69 per cent were females and 31 per cent were males. The most common age represented was the fourth decade and the average duration of the urticaria before consultation was about four years. In regard to personal allergic history—about 15 per cent of the patients had hay-fever, asthma or some other allergic condition, but if the allergic group described above be ignored, only 8 per cent would have a positive history, and this figure approximates the allergic morbidity of the general population. On the other hand, the family history, or allergic background, is more striking, approximating 28 per cent for the

group, a figure that is not materially altered by dropping the allergic group.

The eosinophile count also deserves mention; it averaged only 1.2 per cent for 54 counts distributed uniformly through all groups.

Skin tests in urticaria are notoriously misleading, inasmuch as the skin reacts readily to many substances unrelated to the urticaria. The average number of skin tests per subject was 46, with an average of 6 positive reactions. About 27 per cent of the entire group gave no reaction to skin tests.

Other features that attracted attention were the blood pressure,

TABLE 1

Analysis of 170 cases of urticaria and angioneurotic oedema

	NUMBER	SEX		MOST COMMON AGE DECADE	AVERAGE DURATION	ALLERGIC HISTORY		EOSINOPHILIA	NEGATIVE SKIN TESTS
		♀	♂			Personal	Family		
	per cent	*per cent*	*per cent*		*years*	*per cent*	*per cent*	*per cent*	*per cent*
Infective*	30	69	31	4th	4.1	10	33	0.9	35
Allergic	20	60	40	4th	4.7	30	33	1.5	5
Psychogenic	18	84	16	4th	3.1	6	20	1.5	26
Endocrine	5	100	0	4th	3.8	0	33	0.6	12
Undetermined	25	58	42	4th	4.1	16	23	1.8	41
Composite		69	31	4th	3.9	15	28	1.2	27

* *Infective group*—including all patients allergic to bacteria.

body weight and gastric acidity. Of 57 blood pressure records, 37 per cent were subnormal and 15 per cent were above normal. Forty-eight per cent of 60 patients were under weight and 15 per cent over weight; 68 per cent of 31 patients had a low gastric acidity or achylia while 13 per cent had an hyperacidity. (Determined by alcohol test meal, table 2.)

Three per cent of the group had positive Wassermann reactions. Of 26 patients who were submitted to basal metabolism tests, two gave low readings, the remainder being in the normal zone.

Many patients noticed exacerbation of their hives associated with infectious, chemical, psychogenic, physiologic and physical phenomena.

Foods lead the list with coryza, drugs, nervousness, menses and thermal stimuli in the order named. Table 3 demonstrates the relative frequency.

There were also several recurring associated symptoms such as nausea and vomiting, dysuria and syncope which appeared with the outbreak of the hives. Their frequency is illustrated also in table 3.

Constipation was frequently present, occurring in 47 per cent of the patients questioned.

Individual group analysis. I. *Urticaria associated with infection (allergic to bacteria).* This sub-group we consider the most important

TABLE 2

Associated clinical findings in 170 cases of urticaria and angioneurotic oedema: Blood pressure, body weight and gastric analysis

	BLOOD PRESSURE, 57 CASES			BODY WEIGHT, 60 CASES			GASTRIC ACIDITY, 31 CASES		
	Low	Normal	High	Low	Normal	High	Low	Normal	High
Infective	5	8	7	12	9	2	6	3	0
Allergic	5	10	0	6	5	2	8	0	1
Psychogenic	6	5	2	6	3	2	5	1	1
Endocrine	2	0	0	2	1	0	0	1	0
Undetermined	3	4	0	3	4	3	2	1	2
Composite	21	27	9	29	22	9	21	6	4
Composite, per cent.	37	48	15	48	36	15	68	19	13

(1) Blood pressure: Classification based on age and normal average of the individual.
(2) Normal weight average: ±10 per cent for height, age and sex.
(3) Gastric analysis: Normal free HCl, 20 to 40 per cent; low free HCl, less than 20 per cent; high free HCl, greater than 40 per cent. Achylia was found in seven cases.

both because it represents the greatest number (52 cases or 30 per cent) of patients and because of the satisfactory response to treatment. The criterion for inclusion in this sub-group is the presence of some focus of infection, the removal of which resulted in permanent disappearance of urticaria within a reasonable period of time. The infections found were:

Chronic tonsillitis	24	Undetermined infections	3
Oral sepsis	20	Acute pharyngitis	1
Sinusitis	9	Prostatitis	1
Cholecystitis	3	Fistula in ano	1
Chronic bronchitis	2	Cervicitis	1

In this group 35 patients underwent operation and 20 were treated with injections of vaccine. The vaccine used was a mixture of equal parts of the following cultures:

Streptococcus (alpha)	Staphylococcus aureus
Streptococcus viridans	Staphylococcus albus
Streptococcus nonhemolyticus	Pneumococcus
Streptococcus hemolyticus	B. coli

These organisms were recovered from the blood stream of infected human hosts, grown twenty-four hours, and killed at 58°C. Tricresol was added to the mixture as a preservative.

TABLE 3

Associated provocative factors in the precipitation of urticarial episodes

	HIVE PROVOCATIVE FACTORS					
	Coryza	Foods	Drugs	Menses	Thermal stimuli	Nervous, emotional worry
Infective	7	4	3	2	3	2
Allergic	2	10	2	0	0	0
Psychogenic	0	2	1	3	1	4
Endocrine	0	1	0	5	1	0
Undetermined	3	7	4	1	3	4
Composite	(7%) 12	(14%) 24	(6%) 10	(9%) 11	(4%) 8	(6%) 10

As controls, patients of the food-sensitive and of the psychogenic groups were treated unsuccessfully with a similar vaccine.

Seventy-four per cent are reported as cured, 12 per cent as improved and 14 per cent as unimproved (table 4).

Had our advice been followed, it is possible that the unimproved 14 per cent (7 patients) would have been reduced. Of these 7 patients 3 had chronic infection of tonsils and adenoids; a fourth patient had chronic tonsillitis and abscessed teeth, but radical treatment was refused. One patient with most persistent constipation did not respond to treatment and the sixth patient had infected tonsils removed, but failed to improve. The seventh case on examination showed so many infections—abscessed teeth, chronic tonsillitis and pansinusitis,

chronic cholecystitis and pyelitis as well as a chronic bronchitis—that treatment was impossible.

The month of onset of urticaria was recorded whenever possible (chart 1). Portrayed graphically this demonstrates an interesting peak for the month of January in each sub-group, save the endocrine group, which is too small numerically to be of significance. This would seem to suggest an association with inclement weather, and the attendant upper respiratory infections, the peak being particularly marked in the infective sub-group. A corroboration of this suggestion is found in the number of patients (13 per cent of the infective group)

TABLE 4

Methods and results of treatment

	TREATMENT			RESULTS			YEARS UNDER OBSERVATION
	Diet	Injection	Operation	Cured	Improved	Unimproved	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
Infective	8	38	67	74	12	14	3.5
Allergic	71	0	8	76	20	3	4.1
Psychogenic	6	16	6	28	44	28	3.7
Endocrine	11	11	0	71	14	14	2.3
Undetermined	9	9	7	50	13	37	5.0
Composite	21	17	25	60	20	20	3.7

who volunteered the information that a coryza caused an exacerbation of urticarial phenomena before the removal of the infected focus.

II. *Allergic group (food and drug sensitive).* This group embraced 20 per cent (35 cases) of the patients in our series. As noted above, this group shows a high incidence of personal allergy. Diagnosis depends upon the development of urticaria following the ingestion or local application (cosmetics) of certain definite substances with relief on their withdrawal. Skin tests were chiefly of corroborative value.

Here elimination diet, avoidance of cosmetics, omission of specific drugs, and the use of sulphur ointment in cases of scabies were the therapeutic measures of choice and results of treatment were good; 76 per cent reported freedom from hives as long as the offending agents were avoided.

The chief factors which caused urticaria in the allergic group were:
1. Foods:

Eggs	Celery
Wheat	Potato
Meat	Tomato
Strawberries	Peas
Oranges	Nuts
Apricots	

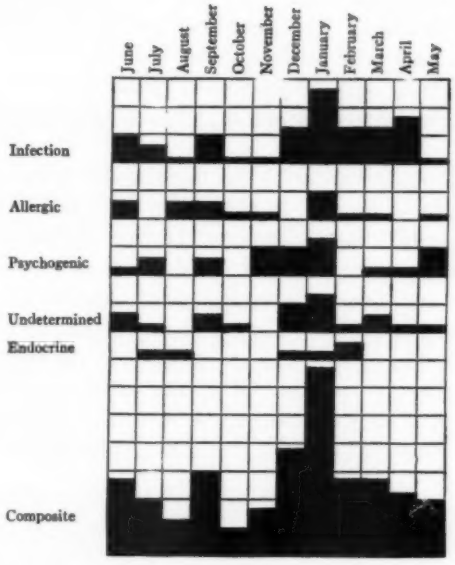

CHART 1. MONTHS OF ONSET OF THE URTICARIAL EPISODE; 95 CASES
1 unit = 3 cases

2. Cosmetics: Lanoline and Cottonseed Oil.
3. Drugs: Cinchophen and Phenolphthalein.
4. Parasites: Scabies.

III. *The psychogenic group.* It is fairly well established that a psychic component is often involved in the causation of hives. The number of times that the observation "nervous temperament" was

made by the examining physician is illustrated in table 3. In this group the nervous or psychogenous element seemed of prime importance; sudden emotional shock or unusual worries would stand in an obvious temporal relationship to the urticarial episode. There was even the occasional patient who said "Just to think of the hives will produce an attack." This group embraced 18 per cent of the total cases of which 84 per cent were in females, and it was the most unsatisfactory group in regard to results of treatment.

IV. *Endocrine group.* This comprises the smallest group with only 9 patients, all females. The conditions represented were as follows:

1. Urticaria only with menstruation—3 cases.
2. Urticaria only with pregnancy—2 cases.
3. Urticaria with hypothyroidism, relieved by glandular therapy—2 cases.
4. Urticaria occurring during the menopause—2 cases.

V. *Undetermined group.* In this group no definite diagnosis was possible for several reasons; some of the patients had a single short attack of hives; in some the cause could not be ascertained in spite of careful study, and some were not sufficiently studied or did not reply to our follow-up inquiries. They probably represent a combination of all the preceding groups, and are included in the series as a sort of control. Twenty-five per cent of all the patients fell in this group and the results of clinical examination in these cases approximate so closely the findings for the entire series that no special discussion seems necessary.

Treatment. Of the group of 170 patients 21 per cent were treated with elimination diets, 25 per cent had foci of infection removed, and 17 per cent received injections of stock vaccine. The rationale of the vaccine therapy was on a non-specific basis, as it was soon ascertained by trial that autogenous vaccine had no advantage over the stock cultures. This treatment was surprisingly effective; temporary relief of several weeks' duration was given to those patients with foci of infection before operation. It is our impression, however, that permanent relief cannot be obtained unless the more radical procedure is carried out (table 4).

The endocrine group cleared up spontaneously, with the termination of pregnancy, excepting the patients whose urticaria was found

to be related to the menstrual period. In several instances the hives ceased to appear after the menopause. Two patients with hypothyroidism were free from hives so long as the thyroid extract was taken.

The psychogenic group required the attention of the psychiatrist; a re-education and adjustment to environmental factors seems to us of the utmost importance. In this sub-group many of our patients could produce flares with slight provocation, as by some emotional upset, and it is our firm belief that our therapeutic results will improve, only when we who are interested in allergic diseases differentiate these patients from the more specific types.

It is our desire to refer especially to the association of infection and urticaria. This relationship is by no means a matter of recent observation, as in 1907 Lustgarten and Winfield (5), in 1923 Barber (6), in 1928 Menagh (7), and in 1929 one of us (L. N. G. (8)), emphasized its importance. Infections were the chief cause of urticaria in our group, and rapid and permanent relief resulted when the infections were removed.

SUMMARY

One hundred and seventy cases of urticaria and angioneurotic oedema studied from two to ten years are reported. Information on the progress of each patient has been obtained during the past two months. The cases can be divided into five simple etiological groups, as follows:

1. Urticaria associated with infection.
2. Urticaria associated with foods, drugs and other extrinsic factors.
3. Urticaria dependent on psychogenic disturbances.
4. Urticaria due to disturbances of the endocrine glands, ovarian or thyroid function.
5. Urticaria of undetermined cause.

Of the entire series 69 were females and 31 males. Twenty-eight per cent gave an antecedent history of allergic phenomena, but personal history of allergy was of no significance. Eosinophilia was not noted.

Fifty-two cases were associated with infections; of these, 74 per cent were permanently relieved by removal of the infected tissue.

Thirty-five cases were due to allergic factors; avoidance of the specific cause permanently relieved these patients of their urticaria.

Treatment of the psychogenic group was less satisfactory. It is our belief that the psychiatrist is far more helpful to these patients than the allergist.

Th group of patients with disturbance of endocrine function responded satisfactorily depending on nature's physiological adjustment, or on small doses of thyroid extract.

We have little to suggest concerning cause or treatment of the "Undetermined groups"—with further study the number will undoubtedly be reduced.

The average duration of symptoms in the entire group before consultation was about four years; the average period of observation since consultation was 3.7 years.

In conclusion, it is our belief that a more optimistic prognosis can be entertained provided a more careful diagnostic study of each patient is made with less emphasis on the importance of allergy *per se*.

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PROTOCOLS

The following protocols are examples of the different groups into which these cases have been classified.

Group I. Urticaria due to infection

Case 1. No. 2455. Age 36. Diagnosis: Urticaria due to abscessed teeth and chronic infection of the tonsils. The history of urticaria continued for six years. Personal allergic history negative, excepting a violent dermatitis on the skin following the use of adhesive tape. Family History: negative. Elimination diet gave

no relief. Examination as far as protein tests are concerned negative. Bacterial tests, intradermal injection, positive to streptococcus viridans. Physical examination showed two abscessed teeth and chronic infection of the tonsils. Removal of the infection resulted in complete elimination of the urticaria. Four years' remission.

Case 2. No. 3508. Age 23. Diagnosis: Urticaria due to infection (abscessed teeth). A history of urticaria for one year. Personal allergic history negative. Family History: Mother suffered with urticaria as a child and as a young adult. Examination as far as protein tests are concerned revealed positive reaction to a number of the grains. Elimination diet gave no relief. Physical examination revealed two abscessed teeth. Relief of urticaria after the extraction of the teeth. No urticaria even though the patient eats the foods to which she is skin sensitive. Two years' remission.

Group II. Urticaria due to foods and drugs

Case 3. No. 1294. Age 27. Diagnosis: Angioneurotic oedema, urticaria due to the ingestion of celery. Personal allergic history—the patient suffers with summer hay-fever due to the pollens of ragweeds. Family History: As far as can be determined negative. The patient gives a history of angioneurotic oedema and urticaria for a number of years. Protein Tests—entirely negative except for a marked reaction to celery and ragweed pollen. Physical examination: All infection had been removed previous to the time of our observation in an effort to relieve her of the urticaria and angioneurotic oedema, but without result. So long as the patient abstains from celery, she is entirely free from symptoms.

Case 4. No. 4483. Age 45. Diagnosis: Angioneurotic oedema and urticaria due to the ingestion of foods (pork). A history of recurring attacks of swelling for four years. Personal allergic history essentially negative. Family History: Negative. Protein tests entirely negative except for marked reaction to pork. Physical examination entirely negative. It was possible to produce angioneurotic oedema and urticaria whenever the patient ate fresh pork, bacon or ham.

Group III. Urticaria due to psychogenic disturbances

Case 5. No. 3556. Age 35. Diagnosis: Urticaria due to psychogenic factors. A history of urticaria and hives of several years' duration. Personal allergic history negative. Family History: Negative. Protein tests revealed positive reactions to a number of vegetables of questionable significance. The patient gives a history of urticaria only when fatigued, under nervous strain or when social activities cause loss of sleep. In her report of March 1934, three years after the consultation, she states that only under these conditions (cited above) does the urticaria return. "Just thinking about it now seems to cause a swelling in my throat, giving me the sensation as if it were closing up."

Group IV. Urticaria due to disturbances of the endocrine glands, ovarian or thyroid function

Case 6. No. 4514. Age 56. Diagnosis: Urticaria and angioneurotic oedema due to disturbance of the endocrine glands (hypothyroidism). A history of angioneurotic oedema for ten years. Personal allergic history essentially negative. Family History: Mother had allergic rhinitis. Protein tests entirely negative. Physical examination was negative except for a low metabolic rate. Vaccine therapy was tried but without relief. Therapy consisted of Thyroid Extract (Hynson, Westcott and Dunning preparation, ½ grain three times a day). The patient reported frequently during the course of treatment and it was found that so long as the basal metabolism was maintained above minus 15, she was free from symptoms.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this journal.)

Bacteriology. For Medical Students and Practitioners. By A. D. GARDNER. 276 pp. $2.25. (*Oxford Univ. Press, New York, 1933.*)

As stated in the preface "The chief aim of this book is to present shortly, readably, and relevantly as much of the vast subject of bacteriology as a medical student or practitioner needs to know . . ." Whether the author has succeeded or not depends upon the varied needs of the student and practitioner. Nevertheless, by a careful study of this little book the reader should acquire a well-balanced perspective of the subject which is much to be preferred to the disorganized pile of information which is likely to be accumulated from our too short courses in bacteriology for medical students. The author has succeeded remarkably well with a very difficult task. The book is not a complete textbook, but if the medical graduate could retain this much and know where to find more, he would be much better informed and better able to utilize the discoveries of bacteriology than are many practitioners.

J. H. B.

Stalkers of Pestilence. By WADE W. OLIVER. 1930. 251 pp. Illust. $3.00. (*Paul B. Hoeber, Inc., New York, 1930.*)

This small volume interestingly but accurately reviews much of the history of medicine from prehistoric times to the present. Although the approach to the subject is biographical, the book is more than a series of brief biographies. Continuity is attained by the tracing of the development of ideas, not ideas which run like threads through the whole fabric but ideas which rather resemble building stones placed one on top of the others until there has emerged a structure quite unlike and transcending the component parts, a structure far from complete, and in the building of which discarded stones may sometimes be seen to have served a useful purpose.

The foreword by Theobald Smith is an excellent preview of the work, and to be fully appreciated must be reread after one has finished reading the book.

J. H. B.

A New Approach to Dietetic Therapy. By EUGENE FOLDES. 434 pp. (*Gorham Press, Boston, 1933.*)

This book reads entertainingly, and, to those not familiar with experimental science, very convincingly. Those familiar with experimental literature will be skeptical of the soundness of the author's views. To critical readers, his inter-

pretation of the significance of water and salt in the etiology of a considerable variety of diseases will seem based on very slender evidence. Experimental studies on both humans and animals show much greater regulatory powers and ability to maintain normal composition of body fluids and tissues than the author's discussion indicates. It is one of those books which contain a kernel of truth with a overworked thesis.

E. V. McC.

The Genius of Louis Pasteur. By PIERS COMPTON. 361 pp. $4.50. (*Macmillan Co., New York, 1932.*)

This recent biography is quite adequate, as including the scientific achievements in the more intimate events in the life of Pasteur, his religious beliefs and other matters.

H. W. J.

A Short Encyclopedia for Nurses. By EVELYN C. PEARCE. 625 pp. $3.75. (*Oxford Univ. Press, New York, 1934.*)

This is a complete, concise and well-arranged quick reference book, which should be very useful to students studying for State Board Examinations, and to the itinerant nurse who has not ready access to a library.

L. E. K.

Hypertension and Nephritis. By ARTHUR M. FISHBERG. 3rd edition. Illust. 668 pp. $6.50. (*Lea & Febiger, Philadelphia, 1934.*)

The general arrangement of this third edition is the same as that of the earlier ones. Many phases of the nephritis problem are considered. There are chapters on the pathological physiology of renal function, one on the hypertensive encephalopathies and another on the hypertensive retinopathies. The principles of Volhard and Fahr are followed in the difficult question of classification. An etiological classification, while desirable, is impossible at this time. The nephropathies are discussed under (1) Benign albuminuria, (2) Nephrosis, (3) Nephritis, (4) Multiple glomerular emboli, as in bacterial endocarditis, (5) Essential hypertension, (6) Senile arteriosclerotic kidney. Many sections have been altered or added, and the book is one of the most complete on the subject in the English language.

H. W. J.

Metabolic Diseases and their Treatment. By ERICH GRAFE. 551 pp. $6.50. 37 engravings. (*Lea & Febiger, Philadelphia, 1933.*)

An excellent translation of a well-known textbook by a recognized authority. It is clearly and attractively written; the material has been carefully selected, and the references are abundant, well-chosen and accurate. It is probably the best short text available at the present time on disorders of metabolism. The sections on obesity and on diabetes mellitus are particularly interesting and stimulating.

G. A. H.